Amendments to the Specification

Please replace the last paragraph on page 1, starting at line 25, with the following amended paragraph:

The CD80 (B7-1) and CD86 ~~(B7)~~ (B7-2) proteins, expressed on APCs, are critical costimulatory molecules (Freeman *et al.* 1991. *J. Exp. Med.* 174:625; Freeman *et al.* 1989 *J. Immunol.* 143:2714; Azuma *et al.* 1993 *Nature* 366:76; Freeman *et al.* 1993. *Science* 262:909). B7-2 appears to play a predominant role during primary immune responses, while B7-1, which is upregulated later in the course of an immune response, may be important in prolonging primary T cell responses or costimulating secondary T cell responses (Bluestone. 1995. *Immunity.* 2:555).

Please replace the first paragraph on page 2, starting at line 1, with the following amended paragraph:

One receptor to which B7-1 and B7-2 9 ~~B7~~ bind, CD28, is constitutively expressed on resting T cells and increases in expression after activation. After signaling through the T cell receptor, ligation of CD28 and transduction of a costimulatory signal induces T cells to proliferate and secrete IL-2 (Linsley, P.S., et al. 1991 *J. Exp. Med.* 173, 721-730; Gimmi, C.D., et al. 1991 *Proc. Natl. Acad. Sci. USA.* 88, 6575-6579; June, C.H., et al. 1990 *Immunol. Today.* 11, 211-6; Harding, F.A., et al. 1992 *Nature.* 356, 607-609). A second receptor, termed CTLA4 (CD152) is homologous to CD28 but is not expressed on resting T cells and appears following T cell activation (Brunet, J.F., et al., 1987 *Nature* 328, 267-270). CTLA4 appears to be critical in negative regulation of T cell responses (Waterhouse et al. 1995. *Science* 270:985). Blockade of CTLA4 has been found to remove inhibitory signals, while aggregation of CTLA4 has been found to provide inhibitory signals that downregulate T cell responses (Allison and Krummel. 1995. *Science* 270:932). The B7 molecules have a higher affinity for CTLA4 than for CD28 (Linsley, P.S., et al., 1991 *J. Exp. Med.* 174, 561-569) and B7-1 and B7-2 ~~B7~~ have

been found to bind to distinct regions of the CTLA4 molecule and have different kinetics of binding to CTLA4 (Linsley et al. 1994. *Immunity.* 1:793). A new molecule related to CD28 and CTLA4, ICOS, has been identified and seems to be important in IL-10 production (Hutloff et al. 1999. *Nature.* 397:263; WO 98/38216). If T cells are only stimulated through the T cell receptor, without receiving an additional costimulatory signal, they become nonresponsive, anergic, or die, resulting in downmodulation of the immune response.

Please replace the paragraph starting at page 2, line 31 and ending on page 3, line 9, with the following amended paragraph:

The importance of the B7:CD28/CTLA4 costimulatory pathway has been demonstrated *in* vitro and in several *in* vivo model systems. Blockade of this costimulatory pathway results in the development of antigen specific tolerance in murine and human**[[s]]** systems (Harding, F.A., et al. (1992) *Nature.* 356, 607-609; Lenschow, D.J., et al. (1992) *Science.* 257, 789-792; Turka, L.A., et al. (1992) *Proc. Natl. Acad. Sci. USA.* 89, 11102-11105; Gimmi, C.D., et al. (1993) *Proc. Natl. Acad. Sci USA* 90, 6586-6590; Boussiotis, V., et al. (1993) *J. Exp. Med.* 178, 1753-1763). Conversely, expression of B7-1 by B7-1 ~~B7 by B7~~ negative murine tumor cells induces T-cell mediated specific immunity accompanied by tumor rejection and long lasting protection to tumor challenge (Chen, L., et al. (1992) *Cell* 71, 1093-1102; Townsend, S.E. and Allison, J.P. (1993) *Science* 259, 368-370; Baskar, S., et al. (1993) *Proc. Natl. Acad. Sci.* 90, 5687-5690.). Therefore, manipulation of the costimulatory pathways offers great potential to stimulate or suppress immune responses in humans.

Please replace the paragraph 4 on page 3, lines 17-18 with the following amended paragraph:

In another embodiment, the step of contacting is performed *ex vivo* ~~ex vivo~~. In another embodiment, the step of contacting is performed *in vivo* ~~in vivo~~.

Please replace the second through fifth paragraphs on page 4, lines 5-31, with the following amended paragraphs:

Figure 1 shows the average proteinuria grade in NZB/NZW F1 female mice with no treatment or combination treatment with anti-B7-1 ~~anti-B7.1~~ and anti-B7-2 ~~anti-B7.2~~. Proteinuria grading is as follows: Grade 0.5 is "trace" proteinuria; Grade 1 equals ~30 mg/dL; Grade 2 equals ~100 mg/dL; Grade 3 equals ~300 mg/dL; Grade 4 equals >2000 mg/dL; and Grade 5 represents death. Clinically significant levels of proteinuria [[is]] are shown by the dotted line.

Figure 2 is a table illustrating the dosing protocol used to treat NZB/NZW F1 (B/W) mice. In particular, the amounts of anti-B7-1 ~~anti-B7.1~~, anti-B7-2 ~~anti-B7.2~~, and Rapamycin are highlighted as well as the duration and timing of the therapy.

Figure 3 is a table illustrating the histopathologic evaluation ~~Evaluation~~ of NZBxNZW F1 hybrid female mice at 42 weeks of age after no therapy or treatment with Rapamycin 8 weeks (weeks 29-36). Histology was graded according to a predetermined arbitrary scale as follows: Normal: 0; Slight=1; Mild=2; Moderate=3; Marked=4; Severe=5; and Focal not Diffuse=( ).

Figure 4 (A) shows survival curves for NZB/NZW F1 (B/W) mice after no treatment, single therapy treatment, early versus late dosing treatment, or combination therapy treatment. Early Rapamycin treatment began at 25 weeks and the late Rapamycin treatment began at 33 weeks. Each group contained ten mice. (B) shows average proteinuria grade for mice subjected to no treatment, single therapy treatment, early versus late Rapamycin treatment, or combination therapy treatment. Average proteinuria was graded as described in Figure 1. Clinically significant levels of proteinuria are [[is]] shown by the dotted line.

Please replace the first paragraph on page 5, starting at line 1, with the following amended paragraph:

The instant invention is based, at least in part, on the finding that agents that decrease co-stimulatory signals to T cells are more efficient in reducing symptoms of autoimmune disease when used in combination with Rapamycin or Rapamycin-like compounds. The instant invention provides improved methods of downmodulating immune responses by a cell expressing a B7 molecule with a combination of at least one antibody which binds to at least one B7 molecule and a Rapamycin compound. In a preferred embodiment, at least two antibodies which bind to at least two different B7 molecules are contacted with a cell expressing a B7 molecule in combination with a Rapamycin compound. The subject methods are useful for downmodulation of unwanted immune responses, e.g., autoimmune responses. The subject methods can be performed either *in vitro* ~~in vitro~~ or *in vivo* ~~in vivo~~. Preferably, the methods of the invention are used to treat systemic lupus erythematosis.

Please replace the first paragraph on page 6, starting at line 1, with the following amended paragraph:

As used herein, the term "costimulate" with reference to activated immune cells includes the ability of a costimulatory molecule to provide a second, non- activating receptor mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal can result in cytokine secretion, e.g., in a T cell that has received a T cell-receptor-mediated signal. As used herein the term "costimulatory molecule" includes molecules which are present on antigen presenting cells (e.g., B7-1, <u>B7-2</u> ~~B7~~, B7RP-1 (Yoshinaga et al. 1999. Nature 402:827), B7h (Swallow et al. 1999. Immunity. 11:423) and/or related molecules (e.g., homologs)) that bind to costimulatory receptors (e.g., CD28, CTLA4, ICOS (Hutloff et al. 1999. Nature 397:263), B7h ligand (Swallow et al. 1999. Immunity. 11:423) and/or related molecules) on T cells. These molecules are also collectively referred to herein as "B7 molecules."

Please replace the third paragraph on page 9, starting at line 8, with the following amended paragraph:

As used herein, the term "extracellular domain of a B7 molecule" includes a portion of a B7 molecule which, in the cell-associated form of a B7 molecule, is extracellular. A B7 extracellular domain includes the portion of a B7 molecule which mediates binding to a costimulatory receptor, e.g., CD28, ICOS, and/or CTLA4. For example, the human B7-1 extracellular domain comprises from about amino acid 1 to about amino acid 208 and the human B7-2 ~~B7-~~extracellular domain comprises from about amino acid 24 to about amino acid 245. See, for example, B7-2 (Freeman et al. 1993 *Science*. 262:909; GenBank Accession numbers P42081 or A48754; or United States Patent 5,942,607); B7-1 (Freeman et al. *J. Exp. Med.* 1991. 174:625; GenBank Accession numbers P33681 or A45803; or United States Patent 5,858,776).

Please replace the paragraph on page 13, starting at line 16 and ending on 14, line 10, with the following amended paragraph:

Using B7 cDNA molecules, peptides having an activity of B7 can be produced using standard techniques. Host cells transfected to express peptides can be any procaryotic or eucaryotic cell. For example, a peptide having B7 activity can be expressed in bacterial cells such as *E. coli,* insect cells (baculovirus), yeast, or mammalian cells such as Chinese hamster ovary cells (CHO) and NS0 cells. Other suitable host cells and expression vectors may be found in Goeddel, (1990) *supra* or are known to those skilled in the art. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari. et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series (Smith et al., (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow, V.A., and

Summers, M.D., (1989) *Virology* 170:31-39). Generally, COS cells (Gluzman, Y., (1981) *Cell* 23:175-182) are used in conjunction with such vectors as pCDM8 (Seed, B., (1987) *Nature* 329:840) for transient amplification/expression in mammalian cells, while CHO (dhfr- Chinese Hamster Ovary) cells are used with vectors such as pMT2PC (Kaufman et al. (1987), *EMBO J.* 6:187-195) for stable amplification/expression in mammalian cells. A preferred cell line for production of recombinant protein is the NS0 myeloma cell line available from the ECACC (catalog #85110503) and described in Galfre, G. and Milstein, C. ((1981) *Methods in Enzymology* 73(13):3-46; and *Preparation of Monoclonal Antibodies: Strategies and Procedures*, Academic Press, N.Y., N.Y). Vector DNA can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, LIPOFECTIN™ ~~lipofectin~~, or electroporation. Suitable methods for transforming host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory textbooks. When used in mammalian cells, the expression vector's control functions are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and most frequently, Simian Virus 40.

Please replace the last paragraph on page 14, starting at line 28, with the following amended paragraph:

In one embodiment, variants of a B7 polypeptide which function as ~~either~~ B7 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a B7 (or B7 ligand) polypeptide for B7 antagonist activity. In one embodiment, a variegated library of B7variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of B7 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential B7 or B7 ligand sequences is expressible as individual polypeptides, or

alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of B7 or B7 ligand sequences therein. There are a variety of methods which can be used to produce libraries of potential B7 or B7 ligand variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential B7 or B7 ligand sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S. A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

Please replace the second paragraph on page 19, starting at line 14, with the following amended paragraph:

Any agent which binds to a B7 molecules ~~molecule(s)~~ may be used in the subject methods and compositions. In one embodiment, antibodies for use in the instant methods bind to at least one B7 molecule. In yet another embodiment, an antibody of the invention binds to only one B7 molecule (e.g., to B7-1 and not to B7-2). Such antibodies are known in the art. For example, The 2D10 hybridoma, producing the 2D10 antibody, has been described (Journal of Immunology. 1994. 152:2105). In addition, for use in combination with an anti-B7-2 antibody, several anti-B7-1 antibodies are known or are readily available (see, e.g., United States Patent 5,869,050). For example, an anti-mouse B7-1 antibody 1G10 has been described (Powers G.D., et al. (1994) *Cell. Immunol.* 153, 298-311) and an anti-human B7-1 antibody 133 is also available (see Freedman, A.S. et al. (1987) *J. Immunol.* 137:3260-3267; Freeman, G.J. et al. (1989) *J. Immunol.* 143:2714-2722; Freeman, G.J. et al. (1991) *J. Exp. Med.* 174:625-631; Freeman, G.J. et al. (1993) *Science* 262:909-911).

Please replace the last paragraph on page 20, starting at line 25, with the following amended paragraph:

Those skilled in the art will appreciate that, instead of using naturally occurring forms of a B7 molecule for immunization, synthetic peptides can alternatively be employed towards which antibodies can be raised for use [[in]] this invention. Both soluble and membrane bound costimulatory molecule or peptide fragments are suitable for use as an immunogen and can also be isolated by immunoaffinity purification as well. A purified form of a B7 molecule protein, such as may be isolated as described above or as known in the art, can itself be directly used as an immunogen, or alternatively, can be linked to a suitable carrier protein by conventional techniques, including by chemical coupling means as well as by genetic engineering using a cloned gene of the ~~a~~ costimulatory molecule.

Please replace the second paragraph on page 23, starting at line 8, with the following amended paragraph:

Such mammal-produced populations of antibody molecules are referred to as "polyclonal" because the population comprises antibodies with differing immunospecificities and affinities for a costimulatory molecule. The antibody molecules are then collected from the mammal and isolated by well known techniques such as, for example, by using DEAE SEPHADEX™ ~~Sephadex~~ to obtain the IgG fraction. To enhance the specificity of the antibody, the antibodies may be purified by immunoaffinity chromatography using solid phase-affixed immunogen. The antibody is contacted with the solid phase-affixed immunogen for a period of time sufficient for the immunogen to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

Please replace the paragraph starting on page 24, line 24, with the following amended paragraph:

(b) A suspension of antibody-producing cells removed from each immunized mammal secreting the desired antibody is then prepared. After a sufficient time, the mouse is sacrificed and somatic antibody-producing lymphocytes are obtained. Antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred, and can be mechanically separated into individual cells in a physiologically tolerable medium using methods well known in the art. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. Rat, rabbit and frog somatic cells can also be used. The spleen cell chromosomes encoding desired immunoglobulins are immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC™), 10801 University Boulevard, Manassas, VA 20110-2209. ~~(ATCC), Rockville, Md.~~

Please replace the third paragraph on page 32, starting at line 13, with the following amended paragraph:

An antibody, or antigen binding portion, of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate

these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning; A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

Please replace the second paragraph on page 33, starting at line 28, with the following amended paragraph:

The nucleic acid sequences of the present invention capable of ultimately expressing the desired antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic and synthetic sequences is presently the most common method of production, but cDNA ~~CDNA~~ sequences may also be utilized (see, European Patent Publication No. 0239400 and Reichmann, L. et al., Nature 332, 323-327 (1988), both of which are incorporated herein by reference).

Please replace the second paragraph on page 40, starting at line 6, with the following amended paragraph:

The subject therapies can be used to treat a variety of immune diseases, e.g., ~~Examples of~~ autoimmune diseases or disorders associated with an inappropriate or abnormal immune response such as ~~include~~ rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, allergies, contact dermatitis, psoriasis, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, multiple sclerosis, allergic encephalomyelitis, systemic lupus erythematosus, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, scleroderma, Wegener's granulomatosis, chronic active hepatitis, myasthenia gravis, Stevens-Johnson

syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, primary juvenile diabetes, dry eye associated with Sjögren's syndrome, uveitis posterior, and interstitial lung fibrosis.